Association of HLA-DR2 with chronic chagasic cardiopathy in a population at Paraná Northeast region, Brazil

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ABSTRACT. Chagas' disease is one of the major problems concerning public health in Brazil and other Latin American countries. Nevertheless, few studies have addressed the genetic susceptibility to this disease. As immune response genes are located into the HLA system, there was a good reason to study the association between HLA antigens and the cardiac form of Chagas' disease. Thirty-five patients and seventy-two normal individuals, living in the State of Paraná northern region, Brazil, were used as test and control groups, respectively. Classical statistical methods were used to compare HLA frequencies between these groups. Data confirmed a positive primary association with HLA-DR2 (48.4%vs12.3%; Pc=0,0011) and a secondary association with HLA-B7 (31.4%vs8.3%; Pc=0.033). In conclusion, a positive association between DR2 and chronic chagasic cardiopathy was demonstrated in the caucasian Brazilian population, thus supporting the hypothesis of the involvement of genetic factors in the susceptibility to the cardiac form of Chagas' disease.

Key words: HLA, Brazil, cardiopathy, susceptibility, Chagas.

RESUMO. Associação de HLA-DR2 com cardiopatia crônica em uma população da região noroeste do Estado do Paraná, Brasil. A doença de Chagas é um dos maiores problemas que afetam a saúde pública no Brasil e outros países latino americanos. No entanto, poucos trabalhos avaliaram a susceptibilidade genética a esta doença. Como genes de resposta imune estão localizados no Complexo de Histocompatibilidade HLA, decidimos estudar a associação entre os antígenos HLA e a forma cardíaca da doença de Chagas, que parece apresentar um componente auto-imune importante. Trinta e cinco pacientes e 72 controles residentes na região noroeste do estado do Paraná foram utilizados neste estudo. Métodos estatísticos clássicos foram usados para comparar as freqüências HLA entre pacientes e controles. Os dados confirmam uma associação primária com HLA-DR2 (48.4%vs12.3%; Pc=0,0011) e secundária com HLA-B7 (31.4%vs8.3%; Pc=0.033). Concluindo, uma associação positiva entre DR2 e cardiopatia chagásica crônica foi demonstrada numa população de brancos brasileiros, reforçando a hipótese do envolvimento de fatores genéticos na susceptibilidade à forma cardíaca da doença de Chagas.

Palavras-chave: HLA, Brasil, cardiopatia, susceptibilidade, Chagas.

Introduction

Chagas' disease is one of the major problems concerning public health in Brazil and other South and Central American countries (Schmuñis, 2000). It is caused by *Trypanosoma cruzi* and can be manifested either in acute or chronic phase, although the acute phase is rarely observed in humans. Most of the chronic chagasic patients present the indeterminate phase, about 10% develop the digestive phase and 30% develop the cardiac phase (Lana and Tafuri, 2000). The cardiac pathogenesis might be related to the reactivity of T lymphocytes induced by *T. cruzi* against host tissular antigens (Abel *et al*., 1997).

Different manifestations of Chagas` disease in different geographical regions, same geographical region and even in the same family, together with increasing evidence of an auto-immune component involvement on the cardiac symptoms development support the hypothesis of genetic predisposition to Chagas' disease (Vermelho *et al.*, 1993; Cunha-Neto *et al.*, 1993).

Nevertheless, few studies have addressed the role of genetic factors in the Chagas' cardiopathy

development. As immune response genes and genes that control the genetic susceptibility to several auto-immune diseases are located in the HLA system (Marsh *et al.*, 2000) a study of the association between HLA antigens and the cardiac form of Chagas' disease seemed desirable.

Material and methods

Patients and controls

Thirty-five Caucasian Brazilian individuals, living in the State of Paraná northern region, Brazil, represent the patients' group. They were diagnosed through clinical-laboratory parameters, including electrocardiogram, X-ray of the thorax, indirect immunofluorescency test, ELISA, and hemoculture. The control group is represented by 72 Caucasian individuals without Chagas history, living in the same geographical region of the patients, and selected according to the patients age and sex.

HLA typing

HLA typing was performed according to the microlymphocytotoxicity method (Terasaki and McClelland, 1964). B-lymphocytes were isolated from total peripheral blood lymphocytes through nylon-wool adherence. Cytotoxicity of the anti-sera was evaluated by cytofluorocromasia (Bodmer and Bodmer, 1977) under an Alpha photo II microscope. Thirty-five patients and 72 controls were typed for HLA Class I antigens. Thirty-one patients and 65 controls were typed for HLA Class II antigens.

Statistical analysis

The classical Chi-square statistical method with Yates correction (Levin, 1978) was applied to compare the frequency of HLA antigens between patients and controls. The P value was corrected by the number of independent comparisons made at the same locus, resulting in the corrected P value or Pc corrected the p values.

The population etiologic fraction (EF) was estimated according to Green (1982).

Results

Frequencies of HLA-Class II antigens patients and controls are listed in Table 1. The only antigen that presented an increased frequency in the patients' group after P correction was DR2 (48.4% Vs 12.3%, P=0.00011, Pc=0.0011). EF was 6.7 for DR2 positive individuals. HLA-DQ2 presented a sensitive decrease in the patients' group, although not statistically significant after P correction (29.0% Vs 50.8 %, P= 0.045, Pc= 0.135).

Table 1. HLA-DR and DQ antigen frequency in a sample of Caucasian Brazilian patients with chronic chagasic cardiopathy (n=31) and controls (n=65)

HLA	Patients %	Controls %	P	Pc
DR1	9,7	16,9	ns	ns
DR2	48,4	12,3	0,00011	0,0011
DR3	12,9	27,7	ns	ns
DR4	32,3	26,2	ns	ns
DR11	32,3	32,3	ns	ns
DR12	6,5	7,7	ns	ns
DR6	9,7	18,5	ns	ns
DR7	19,4	32,3	ns	ns
DR8	6,5	7,7	ns	ns
DR9	6,5	3,1	ns	ns
DR52	67,7	78,5	ns	ns
DR53	58,1	53,8	ns	ns
DQ1	61,3	53,8	ns	ns
DQ2	29,0	50,8	0,045	ns
DQ3	54,8	52,8	ns	ns

ns = non-significant at 0.05 level; P= probability; Pc= corrected probability = P x 10

Frequencies of HLA-Class I antigens were not different after p correction, except for B7 (31.4% Vs 8,3%, P=0.0022, Pc=0.033). A slight increase of B16, not statistically significant after p correction (25.7% Vs 8.3%, p=0.031, Pc= 0.465), has also been detected (Table 2).

Table 2. HLA-A, B and Cw antigen frequency in a sample of Caucasian Brazilian patients with chronic chagasic cardiopathy (n=35) and controls (n=72)

HLA	Patients %	Controls %	P	Pc
A1	31.4	18.1	ns	ns
A2	51.4	41.7	ns	ns
A3	25.7	20.8	ns	ns
A23	0.0	5.6	ns	ns
A24	17.1	16.7	ns	ns
A25	0.0	1.4	ns	ns
A26	11.4	22.2	ns	ns
A11	8.6	13.9	ns	ns
A28	2.9	4.2	ns	ns
A29	2.9	11.1	ns	ns
A30	2.9	9.7	ns	ns
A31	0.0	1.4	ns	ns
A32	5.7	9.7	ns	ns
A33	8.6	2.8	ns	ns
B5	17.1	26.4	ns	ns
B7	31.4	8.3	0.0022	0.033
B8	20.5	6.9	ns	ns
B44	22.9	19.4	ns	ns
B13	11.4	11.1	ns	ns
B14	14.3	12.5	ns	ns
B15	0.0	5.6	ns	ns
B16	25.7	8.3	0.031	ns
B17	2.9	12.5	ns	ns
B18	5.7	8.3	ns	ns
B21	8.5	6.9	ns	ns
B22	5.7	5.6	ns	ns
B27	0.0	4.2	ns	ns
B35	17.1	33.3	ns	ns
B40	11.4	9.7	ns	ns
Cw1	31.4	18.1	ns	ns
Cw2	5.7	9.7	ns	ns
Cw3	48.6	34.7	ns	ns
Cw4	20.0	15.3	ns	ns
Cw5	5.7	5.6	ns	ns
Cw6	5.7	18.1	ns	ns
Cw7	28.4	22.2	ns	ns
Cw8	0.0	1.4	ns	ns

ns = non-significant at 0.05 level; P= probability; Pc= corrected probability = P x 15

HLA-B7 and HLA-DR2 are genetically linked in most populations studied worldwide (Imanishi *et al*., 1991). Stratified analysis confirms that the HLA-B7 association is secondary to the DR2 association (Table 3).

Table 3. HLA-B7 and DR2 stratified analysis of chronic chagasic cardiopathy patients and controls.

HLA antigen included	HLA antigen excluded	P	Pc
HLA-B7	HLA-DR2	0.0065	ns
HLA-DR2	HLA-B7	0.00037	0.0037

ns = non-significant at 0.05 level; P= probability; Pc= corrected probability

Discussion

The role of major histocompatibility antigens in Chagas' disease development has been demonstrated in a mouse model, in which a C57Bl/10-H2b line is resistant, while the congenital C3H/An-H2K is susceptible to the disease (Trischmann and Bloom, 1982). Moreover, Juri *et al.* (1990) showed that genes of the H-2 complex control both antibody production and acute infection by *T. cruzi*.

A few previous studies in humans have shown heterogeneous results. Llop *et al.* (1991), studying a Chilean population, found a positive association with B40 while Fernandez-Mestre *et al.* (1998) and Nieto *et al.* (2000) found negative associations with DRB1*14 in Southern Peruvian and Venezuelan populations. Previous studies in Brazilian populations found either no association (Fae *et al*., 2000) or a positive association with A30 and DQ1 and negative association with DQ7 and DQB1*106 (Deghaide *et al*, 1998).

The data presented in this paper suggest a positive association between the cardiac form of Chagas' disease and HLA-DR2 antigen in the Brazilian population of the Northern region of Paraná State. EF indicates that about 6.7 % of cardiac Chagas' cases may be attributed to the HLA-DR2 antigen.

Moreover, our data showed a sensitive decrease of the antigen HLA-DR6 among the patients, although not statistically significant at 5%. As for Fernandes-Mestre *et al.* (1998) and Nieto *et al.* (2000) studies, this could be explained by a negative association with DRB1*14, a split of DR6. Patients and controls will be retyped by molecular biology to confirm the DR6 assignments and to define their splits.

Concluding, present data suggest that HLA-Class II antigens play a role in Chronic Chagasic Cardiopathy development in the Brazilian population.

References

ABEL, LC. *et al*. Molecular mimicry between cardiac myosin and *Trypanosome cruzi* antigen B13: identification of a B13-driven human T cell clone that recognises cardiac myosin. *Braz. J. Med. Biol. Res.*, Ribeirão Preto, v.30, n.11, p.1305-1308, 1997.

BODMER W.F.; BODMER J. Cytofluorochromasia. In: RAY, J.G. *et al*. (Ed.). *NIH Manual of tissue typing techniques.* DHEW Pub. (NIH), 1977, p31-34.

CUNHA-NETO E. *et al.* Molecular identification of myosin-cross-reactive Tc 140/116 *T. cruzi* protein: cross reactive antibodies show 100% association with cardiomyopathy patients. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, 88 (Suplem.), p.186, 1993.

DEGHAIDE N.H. *et al*. HLA class I and II profiles of patients presenting with Chagas' disease. *Dig. Dis. Sci.*, New York, v.43, n.2, p.246-352, 1998.

FAE K.C. *et al.* HLA and Beta-myosin heavy chain do not influence susceptibility to Chagas' disease cardiomyopathy. *Microbes Infect*., Buenos Aires, v.2, n.7, p.745-751, 2000.

FERNANDEZ-MESTRE M.T. *et al*. Influence of the HLA class II polymorphism in chronic Chagas' disease. *Parasite Immunol*., Oxford, v.2, n.4, p.197-203, 1998.

GREEN A. The epidemiological approach to studies of association between HLA and disease. II. Estimation of absolute risks, etiologic and preventive fraction. *Tissue Antigens*, New York, v.19, n.4, p.259-262, 1982.

IMANISHI, T. *et al*. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: TSUJI, K. *et al*. *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference.* New York: Oxford Science, 1992. p.1065-1127.

JURI M.A *et al*. Non-litic antibodies in H-2 controlled resistance to acute infection with *Trypanosoma cruzi*. *Braz. J. Med. Biol. Res.*, Ribeirão Preto, v.23, n.8, p.685-695, 1990.

LANA, M.; TAFURI, W.L. *Trypanosoma cruzi* e Doença de Chagas. 10. ed. In: MELO, A.L. *et al*. (Ed.). *Parasitologia Humana*. São Paulo: Atheneu, 2000. cap.11, p. 73-96.

LEVIN J. *Estatística aplicada às ciências humanas*. São Paulo: Harbra, 1978.

LLOP E. *et al*. HLA antigens in Chagas' cardiomyopathy: new evidence based on a case-control study. *Rev. Med. Chile*, Santiago, v.119, n.6, p.633-636, 1991.

MARSH, S.G.E. *et al*. *The HLA Facts Book*. London: Academic Press, 2000.

NIETO A. *et al*. HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection. *Tissue Antigens*, New York, v.55, n.3, p.195-198, 2000.

SCHMUÑIS, G.A. A Tripanosomíase americana e seu Impacto na Saúde Pública das Américas. 2 ed. In: BRENER, Z. *et al*. *Trypanosoma cruzi e Doença de Chagas.* Rio de Janeiro: Guanabara Koogan, 2000. cap.1, p. 1-15.

TERASAKI P.I.; MCCLELLAND J.D. Microdroplet assay of human serum cytotoxins. *Nature,* London*,* v.204, p.998-1000, 1964.

TRISCHMANN J.M.; BLOOM B. R. Genetics of murine resistance to *Trypanosoma cruzi*. *Infect. Immun.*, Washington D.C., v.35, n.2, p.546-551, 1982.

VERMELHO A. B. *et al*. Cross-reactivity between heart muscle cells and *Trypanosoma cruzi* proteins. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v.88, (Suplem.), p. 187, 1993.

Received on March 06, 2002.
Accepted on May 15, 2002.